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(54) Title: METHOD FOR STERILIZING PRODUCTS

(57) Abstract

A method for sterilizing products to remove biological contaminants such as viruses, bacteria, yeasts, molds, mycoplasmas and parasites is disclosed. The method involves providing the product in a form that contains less than 20 % solids and subsequently irradiating the product with gamma irradiation over an extended period of time. Generally the product is irradiated for a period of not less than 10 hours. The extended irradiation time, in conjunction with the low level of solids in the product substantially reduces the damage to the product. The method is useful in sterilizing sensitive materials such as blood and blood components.

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METHOD FOR STERILIZING PRODUCTSFIELD OF THE INVENTION

The present invention relates to a method for sterilizing products to remove biological contaminants such as viruses, bacteria, yeasts, molds, mycoplasmas and parasites.

BACKGROUND OF THE INVENTION

Several products that are prepared for human, veterinary or experimental use may contain unwanted and potentially dangerous contaminants such as viruses, bacteria, yeasts, molds, mycoplasmas and parasites. Consequently, it is of utmost importance that such products are determined to be contaminant free before they are used. This is especially critical when the product is to be administered directly to a patient for example in blood transfusions, organ transplants and other forms of human therapies. This is also critical for various biotechnology products which are grown in media which contain various types of plasma and which may be subject to mycoplasma or other viral contaminants.

Previously, most procedures have involved methods that screen or test products for a particular contaminant rather than removal of the contaminant from the product (1). Products that test positive for a contaminant are merely not used. Examples of screening procedures include the testing for a particular virus in human blood from blood donors. However, such procedures are not always reliable and are not able to detect the presence of viruses in very low numbers. This reduces the value or certainty of the test in view of the consequences associated with a false negative result. False negative results can be life threatening in certain cases, for example in the case of Acquired Immune Deficiency Syndrome (AIDS). Furthermore, in some instances, it can take weeks, if not months, to determine whether or not the product is contaminated.

More recent efforts have focused on methods to remove or inactivate contaminants in the products. Such methods include heat treating, filtration, addition of chemical inactivants and gamma irradiation (2). It is well documented that gamma irradiation is effective in destroying viruses and bacteria (2,3). In fact, one author concludes that gamma irradiation is the most effective method in reducing or eliminating levels of viruses (2). However, when applied to radiation sensitive products, such as blood, gamma irradiation can also have damaging effects on the product itself. In particular, it has been shown that high radiation doses are injurious to red cells, platelets and granulocytes (3).

SUMMARY OF THE INVENTION

In view of the above, there is a need to provide a method of sterilizing products that is effective in removing biological contaminants while at the same time having no adverse effect on the product. Examples of contaminants include viruses, bacteria, yeasts, molds, mycoplasmas and parasites.

Accordingly, the present invention provides a method for sterilizing a product comprising:

- a) providing a product to be sterilized in a form having a solid content of less than 20% by weight; and
- b) irradiating the product for a period of time of not less than 10 hours at a rate sufficient to provide a total dose of irradiation of between about 20 to about 32 kGy.

By the method of the present invention, the gamma radiation is delivered over an extended period of time which substantially reduces the damage to the product. Typically, the irradiation is carried out for a period of time of not less than 10 hours, preferably from about 20 to about 40 hours, more preferably from about 20 to about 30 hours. The rate of irradiation is in the range of from about 0.5 kGy/hr to about 3.0 kGy/hr,

depending on the product to be sterilized as well as the length of the irradiation time. The total amount of irradiation given is typically in the range of from about 20 to about 32 kGy as these levels have been shown to be effective in destroying contaminants such as viruses.

The product is irradiated in a form containing preferably less than 20% solids. Consequently, certain products must be diluted before irradiation. Treating products in diluted form also serves to reduce degradation of the product during irradiation. The choice of diluent depends on the nature of the product to be irradiated. For example, when irradiating blood cells one would choose a physiologically acceptable diluent such as citrate phosphate dextrose.

The process according to the present invention can be carried out at ambient temperature and does not require the cooling, freezing or chemical treatment of the product before the process is carried out. This avoids some of the extra treatment steps that are present in prior art processes.

The method of the present invention is useful in treating organic products that are sensitive to irradiation. Such products may be prone to degradation when irradiated by standard methods. However, irradiating sensitive products by the present method would not be expected to be harmful to the products. The method is typically applied to biological products such as blood and blood components although it is not limited thereto.

In cases where living cells (such as blood cells) are to be irradiated, a scavenger may be added to bind free radicals and other materials that are toxic to cells. A suitable scavenger is ethanol.

The efficacy of the method of the present invention is contrary to what others skilled in this area have predicted. In particular, in United States Patent No. 4,620,908(1) it is stated that if gamma irradiation

was conducted on protein material at ambient temperature, the material would be almost completely destroyed or destroyed to such an extent so as to render the material virtually ineffective. In contrast, when tested on blood, the method of the present invention has not destroyed the viability of the cells contained therein.

DESCRIPTION OF PREFERRED EMBODIMENTS

The following examples are provided in order to illustrate the method of the present invention and are not meant to limit the scope of the invention.

EXAMPLE 1

Sterilization of Blood

A 200 ml bag of one day old packed red blood cells was used. Ethanol was added to the cells in order to achieve a final ethanol concentration of 0.01%. The red blood cells were diluted by a factor of one in ten using a modified Citrate Phosphate Dextrose (CPD) solution having a pH of about 6.4 to 6.7 and having the following composition in a total volume of 500 ml:

Citric Acid Monohydrate	0.2 g
Sodium Citrate Dihydrate	26.3 g
Sodium Monobasic Phosphate	2.2 g
Sodium Dibasic Phosphate	1.0 g
Dextrose	3.2 g

The cells were irradiated in a commercial size gamma irradiator which contained a cobalt 60 source rack. Irradiation was done off carrier in an unprotected box. The cells were irradiated for twenty four hours at a rate of approximately 1 kGy/hr. After the irradiation period the red blood cells were examined visually and were found to be viable, having a brilliant red colour. A control sample, consisting of packed red blood cells that were not

diluted with the above-described CPD solution, was not viable after irradiation.

Four days after the irradiation procedure, the diluted cells were tested for levels of various blood components and the results are shown in Table 1. The control sample consisted of blood from the same bag as the test sample but it did not undergo irradiation. Table 1 illustrates that dilution and irradiation of human blood cells did not significantly alter the white blood cell count. The platelet count a hematocrit values were slightly lower than the control, however these values are still within the range that is seen in normal adult blood. The level of haemoglobin was higher than in the control indicating that some red blood cells did lyse during the procedure. This also evidenced by the lower red blood cell count. Nevertheless, contrary to what has been previously published, up to 25 kGy of radiation did not destroy the components of blood by the present procedure. The cells were also counted and found to be viable after 25 kGy of gamma irradiation.

Table 1

<u>Component</u>	<u>Irradiated Blood</u>	<u>Control Blood</u>
White Blood Cells	4 K/mm ³	4.8K/mm ³
Red Blood Cells	3 Mi/mm ³	7.2 Mi/mm ³
Haemoglobin	42 g/dl	21 g/dl
Hematocrit	46%	64%
Platelet	100 k/mm ³	120 k/mm ³

EXAMPLE 2

30 Sterilization of Dextrose

Dextrose (or glucose) containing solutions are used in the treatment of carbohydrate and fluid depletion, in the treatment of hypoglycaemia, as a plasma expander, in renal dialysis and to counteract hepatotoxins (4,5). Dextrose is also the preferred source of carbohydrate in parental nutrition regimens (4,5). In all of the above

applications, the dextrose must be sterilized before use. Sterilization of dextrose containing products is generally done by heat sterilization or autoclaving. Unfortunately, these methods have been reported to degrade or caramelize dextrose containing solutions resulting in a color change in the solution (5). Gamma irradiation of glucose has also been reported to decompose glucose containing solutions (6). Therefore, there is a need for a method that can sterilize dextrose containing products that does not degrade the product itself. In view of the problems of the prior art, a dextrose solution was treated according to the method of the present invention as follows.

A 5% dextrose solution was irradiated for 24 hours, at a rate of approximately 1 kGy/hr. After irradiation the product was tested and it was found that there was no visible light spectrum change as compared to the non-irradiated control. Therefore, the present method can be useful in sterilizing products that contain dextrose.

Subsequent to the above experiment fresh solutions of 5 and 50% Dextrose were irradiated to 25 kGy over 36 hours at ambient temperature. The results were similar to those described above. In addition, UV/VIS scans were obtained and demonstrated a complete absence of the peak at 283.4 nm for 'furfurals' as per U.S.P. In contrast, dextrose samples sterilized using an autoclave contain the 283.4 furfural peak.

30 EXAMPLE 3

Sterilization of Human Serum Albumin

Normal Human Serum Albumin was irradiated as the 25% salt solution to a total dose of 25 kGy over 36 hours using a Gamma cell 220. The temperature was not controlled during the irradiation but it is estimated that the container holding the albumin solution was

approximately 23°C. The results of HPLC analysis are given in Table 2.

TABLE 2

PARAMETER	CONTROL (%)	IRRADIATED (%)
Polymer	2	3
Dimer	7	8
Monomer	90	86
Low Molecular Weight	1	3
pH	7.05	6.97
NTU (must be > 20)	11.4	11.4

As the results demonstrate, Normal Human Serum Albumin can safely be irradiated to 25 kGy at room temperature without adversely affecting the essential properties of the protein. This has not been demonstrated before. All other attempts at irradiating serum albumin require that it be irradiated in the frozen stage. This adds to the cost and difficulty of doing the irradiation.

EXAMPLE 4

Normal human blood from a healthy donor was taken in a heparinized tube; washed three times with standard CPD solution, then diluted 1:20 with CPD containing 0.01% v/v Ethanol. This latter solution of CPD with 0.01% v/v Ethanol is called SCPD. Two ml aliquots were then placed in 10 ml plastic test tubes and irradiated to different doses to 26 kGy over 36 hours at room temperature. There was no haemolysis and the cells appeared intact if somewhat large and slightly irregular in shape. The results of three separate experiments are reported in Table 3.

TABLE 3

PARAMETER	RBC ¹	HGB ²	HCT ³	MCV ⁴	MCH ⁵	MCHC ⁶	RDW ⁷	FLAGS
1* CONTROL	1.08 .99	41 33	.097 .089	89.5 90.2	38.3 33.0	427 366	17.7 15.3	Nearly Normal
2*				95.0	32.3	339	12.0	
12 kGy 1	1.22 1.38	45 45	.166 .199	135.8 144.7	36.5 33.0	269 228	27.3 24.9	1+Anisocytosis 3+Macrocytosis
1 16 kGy	1.04 0.54	32 29	.169 .088	163.0 162.5	31.3 54.5	192 335	18.8 18.8	1+Anisocytosis 3+Macrocytosis
2	0.82 0.81	27 26	.128 .124	156.5 152.6	32.8 32.4	209 212	19.8 20.2	2+Anisocytosis 3+Macrocytosis
1 20 kGy	0.79 1.26	244 28	.125 .203	158.4 161.5	30.8 22.1	194 137	19.4 19.0	1+Anisocytosis 3+Macrocytosis
2	0.93 0.92	30 30	.141 .143	151.5 155.5	32.3 32.1	213 207	20.1 20.5	2+Anisocytosis 3+Macrocytosis
26 kGy 1	1.15 1.15	34 34	.180 .176	155.9 153.0	29.4 29.9	189 195	19.3 23.4	1+Anisocytosis 3+Macrocytosis

* Experiment 1 and Experiment 2

1. Red Blood Cell Count: Cells x 10¹²/liter
2. Hemoglobin : grams/liter
3. Hematocrit
4. Mean Corpuscular Volume : Femtoliters
5. Mean Corpuscular Hemoglobin : picograms
6. Mean Corpuscular Hemoglobin Concentration : grams/liter

There was no haemolysis and the cells appeared intact if somewhat large and slightly irregular in shape. The cells were easily put into suspension and reconstituted in fresh buffer.

5 The following three experiments (examples 5, 6 and 7) were conducted in order to determine the efficacy of the method when treating HIV-containing blood. In each example the cells were similarly treated. Analysis was done by a second independent laboratory. The second
10 laboratory was also chosen because it could handle the AIDS virus. In these experiments, the cells were gently agitated after 12, 16 and 24 hours. Further, in the third experiment, Example 7), the cells were placed in T25
15 flasks to provide greater surface area and reduce the concentration due to settling in the bottom of the centrifuge tubes.

EXAMPLE 5

Sterilization of HIV-containing Blood

20 The following experiment was undertaken with the following specific objectives:

1. To evaluate the toxicity of the process towards red blood cells (RBCs).

2. To evaluate the anti-retroviral activity of the
25 process.

PROCEDURE

Initially, 2 ml of anticoagulated blood was obtained from an HIV-seronegative donor. The blood was centrifuged, and the plasma was removed. The remaining
30 cell pellet was resuspended in 10 ml of the CPD buffer, and centrifuged. This washing process was repeated a total of three times. The final pellet was resuspended in 40 ml of the SCPD buffer, and distributed into plastic tubes in 2 ml aliquots, with 16 separate aliquots being
35 retained for further manipulation. For 8 of these tubes, an aliquot of HTLV-IIIB was added. This is a laboratory

strain of the HIV virus, and 100 tissue culture infective doses (TCID) was added to each of the tubes to be infected. For the remaining 8 tubes, a "mock" infection was performed, by adding a small amount of non-infectious laboratory buffer, phosphate buffered saline (PBS). Four
5 infected and four non-infected tubes were subjected to the process. For comparison, the remaining 8 tubes (four infected and four non-infected) were handled in an identical manner, except that they were not subjected to
10 the process.

It should be stated that at the beginning of the study, a separate aliquot of blood was obtained from the donor. This was processed in the clinical hematology laboratory and a complete hemogram was performed. These
15 baseline results were compared to repeat testing on the study aliquots, which included evaluation of four processed and four unprocessed samples, all of which were not infected with HIV.

An aliquot of 0.5 ml of each of the infected
20 study samples was inoculated on mononuclear cells (MCs) which had been obtained three days earlier. These cells had been suspended in RPMI culture medium, with 10% fetal calf serum and other additives (penicillin, streptomycin, glutamine, HEPES buffer) along with 1 ug/ml PHA-P. At the
25 same time as this inoculation, the cells were resuspended in fresh medium with rIL-2 (20 U/ml). The cultures were maintained for 7 days. Twice weekly, a portion of the culture medium was harvested for the measurement of HIV p24 antigen levels (commercial ELISA kit, Coulter
30 Electronics, Hialeah, FL) for the measurement of viral growth.

A separate aliquot of the eight infected study samples was used for viral titration experiments. Briefly, serial four-fold dilutions of the virus-
35 containing fluids (ranging from 1:16 to 1:65,536) were inoculated in triplicate in 96-well flat-bottom tissue

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culture plates. PHA-stimulated MCs were added to each well (4 million cells in 2 ml culture medium, with IL-2). An aliquot of the supernatant from each culture well was harvested twice weekly for the measurement of HIV p24 antigen levels. A well was scored as "positive" if the HIV p24 antigen value was > 30 pg/ml. The viral titer was calculated according to the Spearman-Kärber method (see ACTG virology protocol manual) using the following equation:

$$M = xk + d[0.5 - (1/n)r]$$

M: titer (in log 4)

xk: dose of highest dilution

d: space between dilutions

n: number of wells per dilution

15 r: sum of total number of wells

RESULTS

Red blood cell parameters for the baseline sample as well as for the unprocessed and processed study samples are shown in Table 4.

20

TABLE 4

Sample/Number	MCV	MCH	MCHC
Baseline	94.5	32.0	339
Unprocessed-1	91.4	34.4	376
Unprocessed-2	90.2	37.9	420
Unprocessed-3	92.1	40.0	433
Unprocessed-4	91.0	40.2	442
Processed-1	133.4	37.8	284
Processed-2	131.5	45.0	342
Processed-3	128.5	38.9	303
Processed-4	131.1	39.4	301

The abbreviations used in Table 4 are explained under Table 3.

As described above, HIV cultures were established using 0.5 ml aliquots of unprocessed and processed study samples. P24 antigen levels (pg/ml) from the study samples on day 4 and day 7 of culture are shown in Table 5.

Table 5

Sample/Number	p24-DAY 4	p24-DAY 7
Unprocessed-1	1360	464
Unprocessed-2	1180	418
Unprocessed-3	1230	516
Unprocessed-4	1080	563
Processed-1	579	241
Processed-2	760	303
Processed-3	590	276
Processed-4	622	203

Finally, one unprocessed sample and one processed sample were selected for the performance of direct viral titration without culture. The results are shown in Table

6.

Table 6

	Sample/Number	Titer (log 10ml)
	Unprocessed-1	1.5
5	Processed-1	0.0

10 The red blood cells were minimally affected by the process, although some reproducible macrocytosis was observed. Although on co-culturing of processed samples, there appeared to be some residual live virus, this was not confirmed by direct titration experiments.

EXAMPLE 6

15 The objective of this experiment was to evaluate the toxicity of the process towards red blood cells in a comprehensive manner.

METHODS

20 For this experiment, 1 ml of anticoagulated blood was obtained from the same HIV-seronegative donor as in the first experiment. The blood was centrifuged, and the plasma was removed. The remaining cell pellet was resuspended in 10 ml of the CPD buffer, and centrifuged. This washing process was repeated a total of three times.
25 The final pellet was resuspended in 20 ml of the SCPD buffer, and distributed into plastic tubes in 2 ml aliquots, with all 10 aliquots being retained for further manipulation. Eight tubes were subjected to the process, while the final two tubes were retained as control,
30 unprocessed tubes. After the processing, all ten tubes were centrifuged, and the resulting pellet was resuspended in 100 ul buffer. A complete hemogram was performed on these reconcentrated study samples.

35 As in the first experiment, a separate aliquot of blood was obtained from the donor when the study sample

was taken. A complete hemogram was performed on this baseline sample. As the study samples were re-concentrated to 33-50% of their original state, more direct comparisons with the baseline sample could be undertaken than were possible in our earlier experiment.

RESULTS

Red blood cell parameters for the baseline sample as well as for the unprocessed and processed study samples are shown in Table 7. The abbreviations used in Table 7 are defined in Table 3.

Table 7

Sample/Number	RBC	HGB	MCV	MCH	MCHC
Baseline	4.76	152	94.9	31.9	336
Unprocessed-1	0.99	33	90.2	33.0	366
Unprocessed-2	1.08	41	89.5	38.3	427
Processed-1	1.15	34	153.0	29.9	195
Processed-2	1.15	34	155.9	29.4	189
Processed-3	1.26	28	161.5	22.1	137
Processed-4	0.79	24	158.4	30.8	194
Processed-5	0.54	29	162.5	54.5	335
Processed-6	1.04	32	163.0	31.3	192
Processed-7	1.35	45	144.7	33.0	228
Processed-8	1.22	45	135.8	36.5	269

There was macrocytosis of the cells which was present in all the processed samples. Comparable haemoglobin levels were measured in the unprocessed and processed samples. The absolute values were appropriate for the residual dilution. The red blood cells are preserved.

EXAMPLE 7

The objective of this example was to verify and expand upon the results obtained in the example 6.

METHODS

For this experiment 5 ml of anticoagulated blood was obtained from the same HIV-seronegative donor as in the first two experiments. The blood was centrifuged, and the plasma was removed. The remaining cell pellet was resuspended in 100 ml of the CPD buffer, and centrifuged. This washing process was repeated a total of three times. The final pellet was resuspended in 100 ml of the SCPD buffer, and distributed in 25 ml aliquots, in T25 tissue culture flasks, with all four aliquots been retained for further manipulation. Two flasks were subjected to the process, while the other two were retained as control, unprocessed flasks. After the processing, the contents of each of the flasks was observed, and a visual determination of the cells capacity to absorb oxygen (turning a brighter red on exposure to ambient air) was made. Following this, the contents of the flasks was aspirated and centrifuged, with the residual pellet resuspended in a small volume of buffer. A complete hemogram was performed on these re-concentrated study samples.

As in examples 5 and 6, a separate aliquot of blood was obtained from the donor when the study sample was taken. A complete hemogram was performed on this baseline sample. As the study samples were re-concentrated to 33-50% of their original state, direct comparisons of a number of specific parameters would be possible with the baseline sample.

RESULTS

On visual inspection, there were no appreciable differences between the processed and unprocessed study samples. Specifically, there appeared to be a uniform distribution of well suspended cells. On exposure to ambient air, the contents of all flasks became somewhat brighter red. No specific quantitative measurements of oxygenation were made.

Red blood cell parameters for the baseline sample as well as for the unprocessed and processed study samples are shown in Table 8. The abbreviations used in Table 8 are defined under Table 3.

5

Table 8

Sample/Number	RBC	HGB	MCV	MCH	MCHC
Baseline	4.75	153	95.0	32.3	339
Unprocessed-1	0.93	30	151.5	32.3	213
Unprocessed-2	0.92	30	155.5	32.1	207
Processed-1	0.82	27	156.5	32.8	209
Processed-2	0.81	26	152.6	32.4	212

10

This experiment was designed to more closely approximate conditions of red blood cells to be transfused into a patient, and was consequently conducted at higher volumes. On a preliminary basis, it does not appear that the process impairs the red blood cells' ability to carry oxygen, although this should be measured more formally. Interestingly, in this experiment, there was no difference in cell size between the processed and unprocessed samples, both being large compared to baseline. This should be repeated. Comparable haemoglobin levels were measured in all the study samples.

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While the examples relate to specific embodiments of the method of the present invention, it is to be appreciated that the method can be used to treat an extremely wide variety of products that require sterilization. The fact that the method has proven effective in blood which is a fragile biological material makes it reasonable to predict that the method can be used on many similarly sensitive products. Examples of other products that may be treated include pharmaceuticals, proteins, nucleic acids, blood components, body fluids (such as cerebral spinal fluid, saliva), liposomes, glucose containing products, cell cultures, fetal bovine

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serum, bone marrow, organs, foods and cosmetics such as shampoos, lotions and creams.

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CLAIMS

1. A method for sterilizing a product comprising:
 - a) providing a product to be sterilized in a form
 - 5 having a solid content of less than 20% by weight; and
 - b) irradiating the product with gamma irradiation for a period of time of not less than 10 hours at a rate sufficient to provide a total dose of irradiation of between about 20 to about 32 kGy.
- 10 2. A method according to claim 1 wherein said process is carried out at ambient temperature.
3. A method according to claim 1 wherein said
- 15 irradiation is provided at a rate of from about 0.5 kGy/hr to about 3.0 kGy/hr.
4. A method according to claim 1 wherein said product is irradiated for a period of time from about 20
- 20 to about 40 hours.
5. A method according to claim 1 wherein said product is irradiated for a period of time from about 20 to about 30 hours.
- 25 6. A method according to claim 1 wherein said product is an organic product.

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7. A method according to claim 1 wherein said product is a biological product.

8. A method according to claim 1 wherein said
5 product is blood or a component thereof.

9. A method according to claim 8 wherein said blood or blood component is first treated with ethanol.

10 10. A method according to claim 9 wherein said ethanol is in a final concentration of 0.01% and said blood or blood product is diluted before irradiation in a physiologically acceptable diluent to achieve a final dilution of at least 1:10.

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11. A method according to claim 10 wherein said physiological acceptable diluent is a modified citrate phosphate dextrose solution having a pH in the range of about 6.4 to about 6.7.

20

12. A method according to claim 1 wherein said product is diluted in step (a) with a citrate phosphate dextrose solution.

25 13. A method according to claim 12 wherein said citrate phosphate dextrose solution contains about .01% v/v ethanol.

14. A method according to claim 1 wherein said product contains dextrose.
- 5 15. A method according to claim 1 wherein said product is a protein.
16. A method according to claim 15 wherein said product is an antibody.
- 10 17. An organic product treated according to the method of claim 1.
18. A biological product treated according to the method of claim 1.
- 15 19. A blood product or component thereof treated according to the method of claim 1.
- 20 20. A product containing dextrose treated according to the method of claim 1.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L2/00 A61L2/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,90 00907 (SALFORD UNIVERSITY BUSINESS SERVICES) 8 February 1990 see claims 1,2,10 ---	1
A	EP,A,0 334 679 (NATIONAL RESEARCH) 27 September 1989 see claims 1,4 ---	1
A	WO,A,91 16060 (CRYOPHARM) 31 October 1991 see page 15, line 3 - line 13; claims 12-16,21,27 ---	1
A	US,A,4 620 908 (VAN DUZER J.P.) 4 November 1986 cited in the application -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 94/00401

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